A MICRO-ELECTRODE AND UNICELLULAR STIMULATION.

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During the summers of 1918 and 1919, while conducting experiments on unicellular organisms and Echinoderm eggs, it was found necessary to construct a micro-pipette that could be more readily and more accurately controlled than could either Barber's[1] pipette or Chambers's[2] modification of the same.

An apparatus was needed with which it was possible, not only to inject definite amounts of fluid, and extract special parts of cells, but also with which these could be electrically stimulated. Such an apparatus was devised, and although it was only in the process of being perfected, nevertheless with it, fluid could be injected and the membrane and other parts of Echinoderm eggs extracted, and these as well as unicellular organisms electrically stimulated. It was possible, for instance, to stimulate different parts of *Vorticella,* and to determine that the contractile substance of the stalk of this special species differed from the contractile substance of the frog's striated muscle fibers, in that, it did not follow the law of "All or Nothing," which was discovered for these muscle cells by Pratt[4] with his very valuable and interesting micro-electrode. The results pertaining to my experiments with these devises will be published in the near future.

At present only the micro-electrode, imperfect though it be, shall be described, in the hope that it will be improved by some one who has the opportunity and is interested in the experimental fields requiring its use.

The micro-electrodes are Barber pipettes, so modified as to be employed for unipolar stimulation. They are glass tubes about twelve centimeters long, six millimeters in diameter, and drawn

[1] From the Woods Hole Marine Biological Laboratory and the University of Kansas.

[1] Barber, M. A., *The Philippine Jr. Sc.*, Sec. Trop. Med., 9, 307.

[2] Chambers, R., *Am. Jr. Phys.*, 1910, p. 189; BIOL. BULL., 1918, 34, p. 121.

[3] Pratt, F., *Am. Jr. Phys.*, 1917–1918–1919, Vol. 43, 44, 49.

out to a bent tip, having a lumen of from three or more microns in the active electrode, and five or more times this in the indifferent one. The other end of the glass pipettes are more or less bent. If they contain mercury, they have a platinum wire soldered into them, and are fitted airtight with rubber tubing and clamps. The rubber tubing and clamp are supported on a pulley that allows this end to be raised or lowered, thus aiding in the adjustment of the mercury in the bent tip.

The pipettes are supported and regulated on the microscopic stage of the Barber pipette holder, and their tips are operated inside of a Barber moist chamber.

The pipettes may contain mercury or some electrolytic solution. Or the indifferent one mercury and the active one a solution, or partly mercury and only the tip a solution. Or both pipettes may contain fine wire. The active one connected with Pratt's glass-coated platinum tip sharpened to a point of 8 microns, and the indifferent one platinum. Or it may be platinum and the active one contain an electrolyte. When non-polarizable, Porter boot electrodes are introduced in the circuit, the pipettes and the dish containing the boots may be filled with sea water. Or by means of pressure applied from the rubber capped end of the pipette, mercury can be forced toward the tip. Then by diminishing the pressure it can be brought back into the capillary a certain distance, drawing after it some of the solution desired from a hanging drop in the moist chamber. An equilibrium is then established that will remain constant as long as the conditions are not changed. Or the active pipette electrode may be filled with mercury either in accordance with Barber's method, or filled under pressure as far as possible and then placed with the indifferent electrode in contact with a mercury hanging drop. If now the circuit from a battery is closed, the current entering the anode traverses the hanging drop and the cathode. At the moment of the establishment of the current, the equilibrium of forces that holds the mercury at a certain point in the capillary is disturbed. The end of the fine thread of mercury moves upward or downward a certain distance owing to a change in sur-

face tension. The direction and distance of the movement depends of course upon the strength and direction of the current introduced into the active pipette. When the mercury is brought to the tip of this, the pressure clamp is closed, the mercury held near the top, which may end in a drop of the same solution that surrounds the organism that is to be stimulated.

If the active electrode be cathodal, a stimulus of a minimal break shock will stimulate, for instance, the stalk or any desired part of a *Vorticella,* that is in the hanging drop, and near contact with the active electrode, and the effect of the stimulation is observed under the microscope.

A diagram of one micro-electrode is shown in Fig. 1. The pipette holder and moist-chamber are omitted in the illustration.

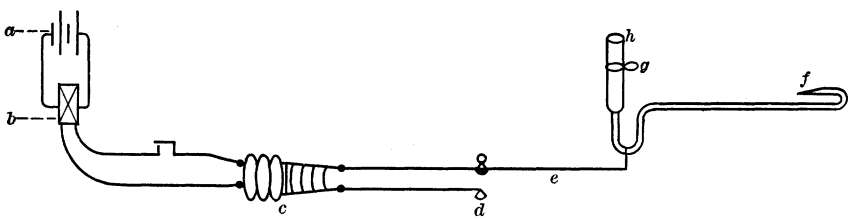

FIG. 1. *a,* battery; *b,* commutator; *c,* induction coil; *d,* clamp; *e,* platinum wire; *f,* tip of pipette; *g,* clamp; *h,* rubber tubing.

The movements of the mercury due to changes in surface tension by a force sufficient to overcome capillary attraction, inaugurated by the passage of an electrical current, made it possible to employ this devise as a capillary electrode.

The fact that the meniscus of mercury moves more or less in either direction in the tip of the micro-pipette by altering the strength and direction of the current, and thus ejecting a solution that lies between the mercury and the tip of the micro-pipette, or drawing in a solution due to suction, led to the idea that it could be adapted for the injection or extraction of minute quantities of substances from unicellular structures. But the mechanism needs further improvements before it can be satisfactorily employed for very accurate work.

I therefore was agreeably surprised while this paper was going

to press, to find that Taylor[1] devised an accurately controllable micro-pipette, that seemed to fulfill the requirements of the apparatus needed for injection and extraction. I believe that by soldering plantinum wire into the capillary containing the mercury and connecting it to batteries, comutator and coil, that it will answer equally well for electrical stimulation.

[1] Taylor, C. V., *Science,* 1920, June 18.